PRODUCTION OF OMEGA-3 FATTY ACIDS FROM *NANNOCHLOROPSIS* SP. ON THE EFFECTS OF CARBON DIOXIDE AND LIGHT INTENSITY

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ABSTRACT

Omega-3 polyunsaturated fatty acid (PUFA) plays a vital role in a number of human health aspects substantially for the regulation of biological function, prevention and treatment of human diseases. Subsequently, humans cannot synthesize the omega-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), thus, intake comes predominantly from dietary. Therefore, the microalgae was discovered as an alternative source of fatty acids compared to fish oil since it offers better purification essential and has no risk of chemical contamination. Moreover, it also acts as a carbon dioxide fixer by photosynthesis process in reducing the atmospheric carbon dioxide. There are major factors give affect for microalgae growth which is carbon source, irradiance level, temperature, nutrient availability and salinity. This study is carried out to investigate carbon dioxide effect in the specific range of 2%, 5%, 10% and 15% at flow rate of 0.2 L/min. Meanwhile, the irradiance levels are examined by using 31 µmol photons m^{-2} $s\text{-}^{1}$, 82 µmol photons m^{-2} s^{-1} , 125 µmol photons m^{-2} s^{-1} and 156 µmol photons m^{-2} $s\text{-}^{1}$ by applying one factor at time (OFAT) in maximizing the production of omega-3 fatty acids. The microalgae of *Nannochloropsis* sp. are cultured in f/2 medium with 300 ml working volume for 7 days of cultivation in an experimental system of photobioreactor. The omega-3 fatty acid compositions of *Nannochloropsis* sp. under different culture conditions are analyzed by lipid extraction and gas chromatography analysis in order to acquire the maximum amount of yield production. The results showed that the highest eicosapentaenoic acid productivity by *Nannochloropsis* sp. had been obtained at 2% of carbon dioxide aeration and 156 µmol photons m^{-2} s^{-1} which are 31.3121 mg/ml and 35.1339 mg/ml, respectively. The fatty acid accumulation of *Nannochloropsis* sp. could be prevailed to produce a high yield under their optimal conditions which are in maximal efficiency of carbon dioxide and high of light intensity.

ABSTRAK

Asid lemak omega-3 memainkan peranan penting dalam beberapa aspek kesihatan terutamanya untuk peraturan fungsi biologi, pencegahan dan rawatan penyakit. Selain itu, manusia tidak boleh mensintesiskan asid lemak omega-3 iaitu, asid eicosapentaenoik dan asid docosahexaenoik, oleh itu, pengambilan asid lemak omega-3 ini diperolehi daripada pemakanan. Disamping itu, mikroalga ditemui sebagai sumber alternatif asid lemak yang penting berbanding minyak ikan kerana ia mempunyai kandungan yang lebih asli dan tidak mempunyai risiko pencemaran kimia. Ia juga bertindak sebagai pengguna karbon dioksida yang berkesan melalui proses fotosintesis bagi mengurangkan kandungan karbon dioksida dalam atmosfera. Terdapat beberapa faktor utama yang memberikan kesan terhadap pertumbuhan mikroalga antaranya ialah sumber karbon, tahap kepekatan cahaya, suhu, nutrien dan kadar kepekatan garam. Kajian ini dijalankan untuk mengkaji kesan parameter karbon dioksida dalam julat peratusan 2%, 5%, 10% dan 15% pada kadar aliran 0.2 L/min. Sementara itu, kesan kepekatan cahaya diuji terdiri daripada 31 µmol photons $m^{-2}s^{-1}$, 82 µmol photons $m^{-2}s^{-1}$, 125 µmol photons $m^{-2}s^{-1}$ dan 156 µmol photons m^{-2} s^{-1} dengan mengaplikasikan satu faktor pada satu masa dalam memaksimumkan pengeluaran asid lemak omega-3. *Nannochloropsis* sp. diternak dalam medium f/2 sebanyak 300 ml selama 7 hari dalam sistem eksperimen photobioreaktor. Komposisi asid lemak omega-3 dalam *Nannochloropsis* sp. di bawah keadaan persekitaran yang berbeza dianalisis melalui kaedah pengekstrakan lipid serta analisis kromatografi gas untuk menentukan hasil pengeluaran yang maksimum. Keputusan menunjukkan bahawa produktiviti tertinggi kandungan asid lemak omega-3 *Nannochloropsis* sp. dihasilkan pada 2% daripada pengaliran karbon dioksida sebanyak 31.3121 mg/ml manakala, 35.1339 mg/ml dihasilkan bagi kesan pengcahayaan pada 156 µmol photons $m^{-2}s^{-1}$. Pengeluaran asid lemak omega-3 yang tinggi daripada *Nannochloropsis* sp. dapat dihasilkan di bawah keadaan optimum iaitu dalam kecekapan maksimum karbon dioksida dan keamatan cahaya yang tinggi.

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PUFAs	Polyunsaturated fatty acids
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
ALA	α-linolenic acid
LA	Linoleic acid
AA	Arachidonic acid
ω	Omega
GRAS	Generally Regarded as Safe
PCBs	Polychlorinated biphenyls
CO_2	Carbon dioxide
GHGs	Green House Gases
sp.	Species
µmol photons m^{-2} s^{-1}	Micromol photons per square meter per second
v/v	Volume per volume
C	Carbon
H	Hydrogen
O	Oxygen
-COOH	Carboxyl group
MUFAs	Monounsaturated fatty acids
LPS	Lipopolysaccharide
PGs	Prostaglandins
LTs	Leukotrienes
NH^{4+}	Ammonium ion
NO_3	Nitrate ion
PO_4^{3-}	Phosphate ion
g	Gram
mg	Milligram
NaCl	Sodium chloride
KCl	Potassium chloride
tris-base	2-amino-2-hydroxymethyl-1,3-propanediol
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$CaCl_2.2H_2O$	Calcium Chloride Dihydrate
$NaHCO_3$	Sodium Bicarbonate
$NaNO_3$	Sodium Nitrate
$NaH_2PO_{4.}H_2O$	Monosodium phosphate monohydrate
$FeCl_3.6H_2O$	Ferric chlorid hexahydrate
Na_2.EDTA	Disodium ethylenediamine tetraacetate
$CoCl.6H_2O$	Hexahydrate of anhydrous $CoCl_2$
$MnCl_2.4H_2O$	Manganese(II) Chloride Tetrahydrate
$CuSO_4.5H_2O$	Copper (II) sulfate pentahydrate
$ZnSO_{4.}7H_2O$	Zinc Sulphate Heptahydrate

Na_2MoO_4	Sodium molybdate dihydrate
ml	Milliliter
ºC/min	Celcius per minute
mL/min	Milliliter per minutes
μd^{-1}	Specific growth rate per day
W	Watt
FID	Flame ionization detector
mm	Millimeter
id	Internal diameter
µm	Micrometer
µL	Microliter
$g\ L^{-1}$	Gram per liter

CHAPTER 1

INTRODUCTION

1.1 Background Study

The dietary omega-3 long chain polyunsaturated fatty acids (PUFAs) have shown increase lipid oxidation and prevent high fat and sugar-induced obesity that help in obesity prevention (Pedersen *et al*., 2011). In addition, to achieve the optimum health, contemporary lifestyles must consider about food choices, eating habits and strategies for building up the omega-3 levels in the body. For individuals ‘at risk’ for diet caused diseases everywhere, even dietary restrictions, prescription remedies, vitamin supplementation, alternative medicine and physical exercise may not be fully protective, preventative or therapeutic without addressing inherent omega-3 fatty acid deficiencies. In other words, the balance of omega fatty acids is important to deliberate. Furthermore, the asserted omega-3: omega-6 ratio has become a model for measuring the proper balance of these fats in oils and the diet. Consequently, omega-3 fatty acids have been focused nowadays in nutrition and medicine especially bioactive lipids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). It has been discovered that certain species of fish and microalgae contain high levels of the essential bioactive omega-3 products EPA and DHA. Meanwhile, the plants also contain diverse levels of omega-3 fatty acids as precursors greatly in the form of α-linolenic acid (ALA). Moreover, there are other dietary long chain fatty acids which are omega-6 fatty acids. Meanwhile, the linoleic acid (LA) is the main omega-6 precursor in plant besides the omega-6 fatty acid arachidonic acid (AA) is bioactive and found in red meat (Doughman *et al.,* 2007).

Accordingly, there are current research suggests increasing accumulated long chain omega-3 fatty acids for health benefits and as natural medicine in several major diseases. Therefore, the first evidence of the important role of dietary intake of omega-3 PUFAs in inflammation was derived from epidemiological observations of the low incidence of autoimmune and inflammatory disorders such as asthma, psoriasis, and type-1 diabetes. The diets containing omega-3 PUFAs as well have also been found to reduce the severity of experimental cerebral and myocardial infarction, to retard autoimmune nephritis and prolong survival of NZB x NZW F_1 mice and also reduce the incidence of breast tumors in rats. Thus, the experimental studies have provided evidence that incorporation of omega-3 fatty acids modifies inflammatory and immune reactions, making omega-3 fatty acids potential therapeutic agents for inflammatory and autoimmune diseases (Simopoulos *et al*., 2002).

Besides that, EPA and DHA from omega-3 fatty acids incorporate into neuronal phospholipids. These omega-3 fatty acids compositions determine the biophysical properties of neuronal membranes and influences neurotransmission. Furthermore, the higher omega-3 PUFAs concentrations lead to higher membrane fluidity, which in turn increases serotonin transport. Dissimilar to *trans*-fats, which have been shown to have negative health consequences, the omega-3 fatty acids are polyunsaturated fatty acids that have been acquainted with many health benefits. In fact they seem to be efficacious in a number of psychiatric and neurological disorders, in particular neurodegenerative diseases. Nevertheless, the omega-3 fatty acids may be helpful in the treatment of dementia and for psychiatric dysfunction in pregnancy and also in breast-feeding (Mazza *et al.*, 2007).

The origin source of omega-3 fatty acids in aquatic ecosystem is microalgae which represent a more direct dietary source of these healthy fatty acids. Besides, there are certain species of microalgae produce high levels of omega-3 fatty acids of EPA and DHA (Doughman *et al*., 2007). The microalgae are able to enhance the nutritional content of conventional food preparations and hence, to positively affect the health of humans and animals. This is regarding to their original chemical composition for example algal lipids that composed of glycerol, from bases esterifies to saturated or unsaturated fatty acids.

Additionally, the microalgae contain many major lipid classes and fatty acids and also constituted as principal producers in the biosphere of some polyunsaturated fatty acids. Besides, the microalgae become current potential sources of these fatty acids and can be provided as a cheap and reliable source to satisfy pharmaceutical requirements (Alonso *et al*., 1998). Whereas, the microalgae are recognized as the primary food source for a large number of aquatic organisms and play a key role in aquaculture development. Therefore, the microalgae also represent a valuable source of nearly all essential vitamins. Otherwise, the microalgae as well as rich in pigments like chlorophyll, carotenoids and phycobiliproteins. Thus, these compositions as represented in Table 1.1 gives microalgae interesting qualities which can be applied in human and animal nutrition (Spolaore *et al*., 2006).

Table 1.1: Particularly interesting microalgal PUFAs

PUFA	Structure	Potential application	Microorganism producer
γ-Linolenic acid (ALA)	18: 3 ω6, 9, 12	Infant formulas for full-term infants Nutritional supplements	*Arthrospira*
Arachidonic acid (AA)	20: 4 ω6, 9, 12, 15	Infant formulas for full-term/preterm infants Nutritional supplements	*Porphyridium*
Eicosapentaenoic acid (EPA)	20: 5 ω3, 6, 9, 12, 15	Nutritional supplements Aquaculture	*Nannochloropsis, Phaeodactylum, Nitzschia*
Docosahexaenoic acid (DHA)	22: 6 ω3, 6, 9, 12, 15, 18	Infant formulas for full-term/preterm infants Nutritional supplements Aquaculture	*Crypthecodinium, Schizochytrium*

Source: Spolaore *et al*., 2006

In fact, the microalgae are unicellular species that has been acknowledged as a primary source of fatty acids. Moreover, some species of microalgae can be actuated to overproduce typical fatty acids through simple manipulations of the physical and chemical condition of the culture medium. As a result of the profound differences in cellular organization and growth modes and the ability to manipulate their fatty acid content, the microalgae represent a significant source of unusual and valuable lipids and fatty acids (Behrens and Kyle, 2007).

Other than that, the microalgae are present in all persisting earth ecosystems, not just aquatic but also terrestrial, which representing a big variety of species living in a wide range of environmental conditions. The microalgae also can act as a nutritional supplement or represent as a source of natural food colorants because of their diverse chemical properties. Hence, many nutritional and toxicological evaluations have been proved about the suitability of algal biomass as feed supplement. Thus, the genetic improvement of algal strains is also a present challenge nowadays but there is a successful drug discovery which is the most promising aspect of microalgae biotechnology because of the potential is immense (Spolaore *et al*., 2006).

The employment of lipids and fatty acids from microalgae as food components required that the microorganisms to be grown at large scale under controlled growth conditions. For growth of microalgae, there is a few relatively simple condition factors have to be met which are light, carbon source, water, nutrients and a suitably controlled temperature. These variety environmental conditions can be adapted by different microalgae species. Consequently, it is possible to find the best suited species of microalgae for certain local environment conditions or specific growth characteristics (Mata *et al*., 2010).

1.2 Problem Statement

Although the fish actually is one of fatty acid source, but there is a classification by The U.S. Food and Drug Administration about intake of up to 3 grams of fatty acids for daily from fish as GRAS (Generally Regarded as Safe). Intake of fish oil for many months may cause a deficiency of vitamin E, and therefore this constituent is added to many commercial fish oil product. Thus, for regular use of vitamin E-enriched products may lead to the elevated levels of this fat-soluble vitamin. Unfortunately, the fish liver oil already contains the fat-soluble vitamins A and D that may increase the content of vitamins and this will contribute to the toxicity of vitamin in the body and cause to the bone fractures, liver damage or even death.

Moreover, the high doses of fish oil have also been associated with nosebleed and bleeding in the urine because it can decrease platelet aggregation, prolong bleeding time and increase fibrinolysis. Besides, the gastrointestinal upset is common with the use of fish oil supplements. The diarrhea may also occur, with potentially severe diarrhea at very high consumption. There are other side effects also report of increased burping, acid reflux, heartburn, indigestion, abdominal bloating, and abdominal pain.

Potentially harmful contaminants such as dioxins, methyl mercury, and polychlorinated biphenyls (PCBs) are found in some species of fish which have been considered as harmful food contaminants. Therefore, by consuming fish is significantly as the main pathway for human exposure to methyl mercury which is one of the most toxic forms of mercury that has been reached relatively high concentrations in most species of fish (Gochfeld and Burger, 2005). Meanwhile, it has been proved that some fish oil capsules sold as health supplements for providing the fatty acids content have illegally undisclosed unnecessarily high levels of contamination with polychlorinated biphenyls (PCBs) compounds. PCBs and methyl mercury are believed to have long half-lives in the body and can accumulate in people who consume fish on a frequent basis. The recommendation currently suggest by limiting intake of fish in dietary. For the case of PCBs, it is suggested that consumers to reduce their exposure to these contaminants by removing the fat from the fish before cooking them, because it is distributed throughout skin, muscle and organs of the fish.

Besides, the global warming issue which is one of the hottest global issues because of the big impact on our universe and environment. The global warming affects an increase in the average temperature of the earth's atmosphere, especially a sustained increase sufficient to cause climatic change. Therefore, carbon dioxide (CO_2) actually is the main greenhouse gas which contributes about 75% of Green House Gases (GHGs) compositions (Purba and Taharuddin, 2010). There are many attempts to recover CO_2 from atmosphere including physical and chemical treatments have been used. The reduction of CO_2 using microalgae as CO_2 fixers can be used as an efficient solution in biological approach (Sheng-Yi *et al.*, 2008). The microalgae contain organic substances such as polysaccharides, lipid, vitamins, minerals, and other bioactive substances. Hence, the photosynthetic CO_2 fixation by microalgae which have higher photosynthetic efficiency is the most effective carbon sequestration method on earth and have been thought to be a feasible technology with energy-saving and environment-friendly (Tang *et al*., 2011).

There is an additional problem by using fish as a source of fatty acid which affects the environmental issue because of carbon dioxide emissions associated due to over-fishing. In addition, over-fishing practices and depletion of some stocks may become so severe that may be regarded as economically extinct happened. Some overfishing practices may deplete populations and unsustainable at ecosystem level (Coll *et al.*, 2008). Hence, the microalgae are used as the new alternative source of fatty acid besides become as a prevention of marine pollution (Venegas-Calerón *et al*., 2010). Consequently, microalgae oil has potential benefits compared to fish oil which are high level content of fatty acids, has lower risk of contamination with heavy metals and more natural taste instead of fishy aftertaste.

1.3 Objectives

To study the effects on carbon dioxide and light intensity in producing high yield of omega-3 fatty acid from the strain of *Nannochloropsis* sp.

1.4 Scope study

To analyze the effect of carbon dioxide concentration in air stream on lipid accumulation of *Nannochloropsis* sp. in order to produce the high quantity of omega-3 fatty acid. The ranges of carbon dioxide percentage used are 2%, 5%, 10% and 15%.

To identify the light intensity required for supporting the carbon fixation through the photosynthesis process of *Nannochloropsis sp.* in maximizing the production of omega-3 fatty acid. There are ranges of irradiance levels which are 31 µmol photons m^{-2} s^{-1}, 82 µmol photons m^{-2} s^{-1} , 125 µmol photons m^{-2} s^{-1} and 156 µmol photons m^{-2} s^{-1} .

1.5 Rationale and Significance

The rationale of this case study is providing the empirical evidence that microalgae have high potential in production of fatty acids. Microalgae have level of oil about 20%–50% (Huang *et al.*, 2010). Production of fatty acid from microalgae has been advanced only in the last decade and has the advantages of lacking unpleasant fish odor, reduced risk of chemical contamination and better purification potential. Therefore, there is the fatty acids from microalgae provide a sustainable and non-contaminated source of these important fatty acids for human nutrition. Thus, not only do these fatty acids help to improve chronic and acute human diseases for examples cardiovascular disease, obesity, type-2 diabetes and metabolic syndrome thus consequently reducing public expenditure on the healthcare system, but also contribute to correct development of neonates and infants (Venegas-Calerón *et al*., 2010).

Additionally, the increasing concentration of greenhouse gases as global issue in the atmosphere has received great concern to world population regarding the matter of global warming. The carbon dioxide (CO_2) which is the principal greenhouse gas, account for 76.7% (v/v), and its concentration have increased rapidly since the onset of industrialization. The anthropogenic emission of CO_2 from coal-fired thermoelectric plants is responsible for up to 7% (v/v) of global CO_2 emissions, meanwhile about 10% to 15% (v/v) of the flue gases emitted from the power plants being in the form of carbon dioxide gas. Thus, microalgae as the photosynthetic microorganisms use inorganic carbon for growth and hence can convert CO_2 from a point source into biomass. CO_2 biofixation method using microalgae is the most important and the most effective carbon sequestration method on earth because of the photosynthetic efficiency, higher biomass production and also faster growth compared to other energy crops. In the applications of microalgae for fixation of carbon dioxide, the tolerance to the CO_2 biomass and fatty acid content of the microalgae are of great important outcome (Tang *et al*., 2011).

Moreover, regarding to the over-fishing activity and concerns about pollution of the marine environment demonstrate a need to develop alternative and sustainable sources of omega-3 fatty acids. Consequently, there is a number of different strategies have been considered, using aquatic organisms as other source of fatty acids. Therefore, regarding to the matter about the sustainability of global fish stocks which actually the main sources of fatty acids because marine fish stocks are in severe decrease as a result of decades of over-fishing. Moreover, environmental pollution of marine ecosystems has resulted in the accumulation of dioxins, content of heavy metals and polychlorinated biphenyls in fish that to be doubtful about the benefits of fish consumption in dietary that will give impact to the human health (Venegas-Calerón *et al*., 2010).

CHAPTER 2

LITERATURE REVIEW

2.1 Fatty Acids

Lipids consist of numerous fat-like chemical compounds that are insoluble in water but soluble in organic solvents. The lipid compounds include monoglycerides, diglycerides, triglycerides, phosphatides, cerebrosides, sterols, terpenes, fatty alcohols, and fatty acids. In addition, lipids are the sole sources of polyunsaturated fatty acids (PUFAs) in microalgae. Besides, the lipid contents significantly are influenced by environmental conditions and can be physiologically manipulated, which results in variation of fatty acid content in the lipid pool (Chen *et al*., 2007). In most cases, lipids are not static but will change actively during normal metabolism or in response to cellular stimuli. The lipids turn over and are biosynthetically remodeled during the cell cycle or experience chemical restructuring to new lipid species with altered properties that serve as bioactive mediators in various signaling pathways. The rapid dynamic change will add to the complexity of lipid metabolism. Furthermore, as an integral building block of semi-permeable membranes, lipids not only function to form barriers between cells and extracellular space and between intracellular organelle compartments but they also affect directly the physical and functional properties of cell membranes (Quehenberger *et al*., 2009).

Concurrently, the fatty acids are merely carboxylic acids with long hydrocarbon chains. The hydrocarbon chain length may vary from 10 to 30 carbons. The non-polar hydrocarbon alkane chain is an important counter balance to the polar acid functional group. Additionally, the fatty acids also consist of the elements carbon (C), hydrogen (H) and oxygen (O) arranged as a carbon chain skeleton with a carboxyl group (-COOH) at one end. The saturated fatty acids (SFAs) have all the hydrogen that the carbon atoms can hold and have no double bonds between the carbons. Moreover, monounsaturated fatty acids (MUFAs) have only one double bond besides polyunsaturated fatty acids (PUFAs) have more than one of double bond. For nomenclature of fatty acids, it begins with the number of carbons, then after a colon, the number of double bonds, followed by the position of the first double bond counting from the omega position in carbon chain. Majority of saturated fatty acids in plasma are palmitic acid (C16:0) and stearic acid (C18:0). Meanwhile, the major monounsaturated fatty acid is oleic acid (C18:1n9), which contains only one double bond. Other than that, there are oleic, stearic and palmitic acids were found in the hydrolysate of the lipid component, which in average about 1.5 % of the lipopolysaccharide (LPS) from the cell wall of microalgae (Mikheyskaya *et al*., 1976).

Nevertheless, it has been found that many of the effects of fatty acids on immune and inflammatory responses are not dependent on eicosanoid generation. Furthermore, the fatty acids have also been found to modulate the process of phagocytosis, reactive oxygen species production, cytokine production, leukocyte migration and also for interfering with antigen presentation by macrophages. The importance of fatty acids in immune function has been corroborated by many clinical trials in which patients which have been showed improvement when submitted to fatty acid supplementation. Several mechanisms have been proposed to explain fatty acid modulation of immune response, such as changes in membrane fluidity and signal transduction pathways, regulation of gene transcription, protein acylation, and also calcium release (Pompeia *et al.*, 2000).

In addition, the fatty acids are important components of other intracellular communication molecules, for examples platelet activating factor, diacylglycerol and ceramides. Despite, even the least sophisticated fatty acids such as the volatile fatty acids or the long-chain saturated fatty acids have important roles in cell metabolism, structure and regulation, with considerable implications in the immune function when the cells in question are leukocytes. Furthermore, the immune system works with specific and nonspecific recognition of foreign molecules, leading to their inactivation or destruction also by specific or nonspecific means. In spite of that, the fatty acids fulfill a variety of roles within immune cells that function as fuels for generation of energy, components of cell membrane phospholipids contributing to the physical and functional properties of those membranes, covalent modifiers of protein structure that affect the cellular, location and function of proteins, regulators of gene expression either through effects on receptor activity, on intracellular signaling processes, or on transcription factor activation and precursors for synthesis of bioactive lipid mediators like prostaglandins (PGs), leukotrienes (LTs), lipoxins and resolvins (Calder, 2008).

There are many types of fatty acids which are divided into two series of essential fatty acids, one has a double bond three carbon atoms removed from the methyl end and the other has a double bond six carbon atoms removed from the methyl end. Several types of fatty acids which are arachidic acid, stearic acid, palmitic acid, erucic acid, oleic acid, arachidonic acid, linoleic acid and linoleic acid as illustrated in Figure 2.1.

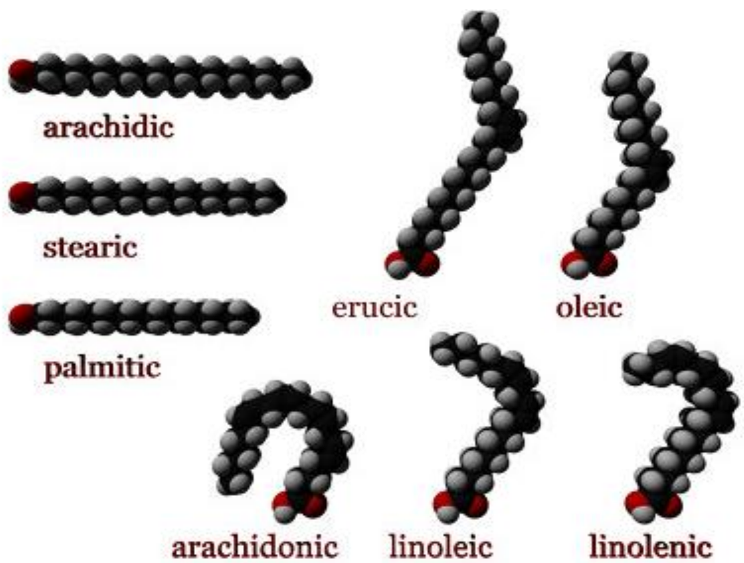

Figure 2.1: Dimensional representations of several fatty acids

Source: biochemistryquestions.wordpress (2008)

Table 2.1: Chemical Names and Descriptions of some Common Fatty Acids

Common Name	Chain Length	Double Bonds	Scientific Name
Arachidic acid	C20	0	eicosanoic acid
Stearic acid	C18	0	octadecanoic acid
Palmitic acid	C16	0	hexadecanoic acid
Myristic acid	C14	0	tetradecanoic acid
Lauric acid	C12	0	dodecanoic acid
Palmitoleic acid	C16	1	9-hexadecenoic acid
Palmitoleic acid	C16	1	*cis*-9-Hexadecenoic acid
Oleic Acid	C18	1	9-octadecenoic acid
Linoleic Acid	C18	2	9,12-octadecadienoic acid
Alpha-Linolenic Acid	C18	3	9,12,15-octadecatrienoic acid
Arachidonic Acid	C20	4	5,8,11,14-eicosatetraenoic acid
Eicosapentaenoic acid	C20	5	5,8,11,14,17-eicosapentaenoic acid
Behenic acid	C22	0	docosanoic acid
Erucic acid	C22	1	13-docosenoic acid
Docosahexaenoic acid	C22	6	cervonic acid

Source: Schroeder and Soler-Argilaga, 1997

Besides, most naturally occurring fatty acids have a chain of an even number of carbon atoms, from 4 to 28. As shown in Table 2.1, the fatty acids are frequently represented by a notation such as C18 that indicates that the fatty acid consists of an 18-carbon chain.

2.1.1 Omega-3 Fatty Acid

Omega-3 fatty acids are important nutrients that are involved in many bodily processes. There are three fatty acids compose in the omega-3 family which are α-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid. The omega-3 fatty acids make up a family of essential fats that humans are unable to synthesize *de novo* and need to be taken in dietary. The primary *de novo* synthesis sources of very long chain PUFAs are marine microbes for example algae which form the base of an aquatic food web that culminates in the accumulation of these fatty acids in the lipids of the fish. Besides, PUFAs consist of 20 carbons or more fatty acids in length with three or more methylene-interrupted double bonds in the *cis* position. Moreover, these fatty acids can be classified into two main families which are omega-6 and omega-3 families, deriving on the position of the first double bond proximal to the methyl end of the fatty acid. PUFAs are also known as vital constituents of human metabolism. In particular, there is plentiful evidence for the health-beneficial properties to humans of dietary consumption of omega-3 PUFAs for example eicosapentaenoic acid (EPA;20:5Δ5,8,11,14,17) and docosahexaenoic acid (DHA;22:6 Δ4,7,10,13,16,19). This dietary requirement is almost certainly due to the fact that humans have limitation of capacity to synthesize these fatty acids from the essential precursor α-linolenic acid (ALA;18:3Δ9,12,15), therefore dietary intake of these fatty acids is a key aspect of human nutrition (Venegas-Calerón *et al.*, 2010).

According to Surette (2008), omega-3 fatty acids are being increasingly promoted as crucial dietary components for health and disease prevention. In addition, there is an increasing number of foods that are not traditional sources of omega-3 fatty acids, such as dairy and bakery products are now being fortified with small amounts of these fatty acids. This recent promotion of omega-3 fatty acids has likely been driven by recommendations for omega-3 fatty acid consumption made by scientific groups such as the American Heart Association. Whence, the research for the molecular and cellular mechanisms by which omega-3 fatty acids affect health and disease has led to a large body of evidence which suggests that these dietary lipids modulate numerous processes, including brain and visual development, inflammatory reactions, thrombosis and carcinogenesis. Simultaneously, the